

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of: Marie Bosnes	)	Examiner: Bhat, Narayan Kameshwar
	)	
Serial No.: 10/501,162	)	Group Art Unit: 1634
	)	
Filed: April 26, 2005	)	Confirmation No.: 7614
	)	
For: Methods for Isolating Nucleic Acids and Proteins from a Single Sample	)	Docket No.: IVGN 819

**Mail Stop Amendment**

Commissioner for Patents

Via EFS-Web

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**DECLARATION BY INVENTOR UNDER 37 C.F.R. § 1.132**

Dear Sir:

I, Marie Bosnes, inventor of the above referenced patent application, hereby declare the following:

1. I am a person of ordinary skill in the art to which this patent application relates.
2. I have reviewed and understand the Final Office Action dated January 28, 2011, and the references cited therein, namely U.S. Patent No. 6,255,477 to Kleiber et al. ("Kleiber"); U.S. 6,723,510 to Lubenow ("Lubenow"); and Schussler et al. (TIG, 1995, 11, 378-379) ("Schussler").
3. In support of the currently pending claims in this case I present the following discussion.

4. Level of skill in the art at the time of the patent application: The time this patent application was written, which was just after the human genome project was completed, it was discovered that humans have fewer genes than expected. The realization that major protein diversity is generated “post-translationally” changed focus to proteomics.

The importance of comparing a gene, its transcripts and protein contents from the same sample became clear to me, since both transcripts and proteins represent a “snapshot” of the cellular situation, both in time and space. Since sample size is often limited, and gene-expression is highly localized (tissue/cell specific), the importance of isolating and analyzing DNA, RNA and protein from the same, small precious sample without dividing it, was the goal of my research.

Since DNA, RNA and proteins have different chemical stability, it was not obvious at that time that different beads could be combined to isolate from one sample all three diverse biomolecules. For example, as is known to one of skill in the art, the binding of bio-molecules to a solid surface is dependent on the interaction between the surface chemistry, the bio-molecular properties and the chemical surrounding buffer system. Different surfaces bind and release in different chemistries.

While it was possible at that time to isolate some species of nucleic acids using certain beads, such as isolating specific mRNAs using oligo(dT) bound to the surface of certain beads or isolating specific proteins using different antibodies or affinity tags on beads, it was not practically possible to combine these methods under similar conditions using the same sample to isolate different species of nucleic acids and proteins. In fact, combining such methods, *i.e.*, combining nucleic acid purification on beads by the methods that existed then and protein purification on beads by methods existing then, would not be possible due to different chemical requirements that could not support the two processes in the same sample.

At the time of my studies described in this patent application there was no known record, to my knowledge, of combining different beads to isolate different species of nucleic acids (*e.g.* DNA and mRNA) and proteins from the same sample. My patent application is the first to demonstrate the isolation of bio-molecules, including different nucleic acid species (DNA and mRNA) and proteins, present in a single cellular sample, using bead

surfaces by using different surface properties of beads and by adjusting the chemical surrounding to achieve attachment of each biomolecule to a different bead, while leaving the other biomolecule unharmed and isolating it using another bead.

5. Why “one of ordinary skill in the art”, at the time the invention was made, would not consider combining Kleiber and Lubenow together to arrive at the claimed invention: Each patent cited by the Examiner and its differences are described in this section:

5a. **Kleiber et al.** describes the problem as to provide better materials for immobilizing biological materials. The subject matter of Kleiber’s invention is:

- magnetic particles with an outer glass surface that is substantially pore-free
- ferromagnetic particles having a glass surface, a procedure for isolating biological materials, especially nucleic acids
- a procedure for the manufacture of magnetic glass particles

All that is described fully and exemplified in Kleiber are the manufacturing process for the particles, the particles themselves and the procedure for isolating nucleic acids in the presence of chaotropic agents.

The Kleiber method involves using particles with a glass surface in combination with chaotropic reagents, which leaves behind denatured proteins. A skilled person would consider such denatured proteins unsuitable for further isolation, and non-functional even if they were somehow isolated. There is indeed no description of how proteins might be purified and in the only relevant example (Example 3), proteinase K is used to digest proteins.

Thus, if anything, Kleiber appears to “teach away” from the presently claimed invention since it teaches the use of “chaotropic agents” and use of proteinase K, both of which would denature or degrade (in the case of proteinase K) protein components from a sample.

Furthermore, Kleiber also writes that “in certain cases the sample can be used without pretreatment in the isolation procedure according to the invention.” Nucleic acids will to my knowledge NOT bind to glass particles unless there are chaotropes and alcohol present.

Despite the reference in Kleiber, at Column 2 lines 1-6, to Streptavidin beads and the Examiner’s allegation in the Final Office Action (at page 10, lines 5-8) that “An artisan having ordinary skill in the art would have a reasonable expectation of success because it merely

involves substituting streptavidin magnetic particles with magnetic particles with ion exchange interactions,” one of ordinary skill in the art will realize that ion exchanger beads are not interchangeable with streptavidin. They both need different specific chemical conditions in order to bind proteins.

In addition, biotinylated proteins (which are the only kind that can bind to streptavidin beads) are not naturally present in cells, and the use of streptavidin beads is only for proteins that are biotinylated (engineered proteins). In contrast, the currently claimed invention is not limited to affinity based protein purification but encompasses isolation of all nucleic acids and all proteins from cells.

Furthermore, Kleiber does not mention anything about the possibility of combining different particles/surfaces to isolate different components from the same sample, sequentially. It merely describes binding of one component, and discarding the rest.

5b. **Lubenow** describes a method to reduce magnetic bead loss, by adding detergent, when isolating a fusion protein by an affinity magnetic bead matrix (immobilized metal ion affinity chromatography, IMAC, using His-tag). It focuses on the potential bead loss and how to minimize that. It suggests adding a detergent like Tween 20 to minimize bead loss which is something that Dynal has long advised customers for “sticky beads” (beads attached to the tube wall and thereby lost).

As described above, in the section describing the level of skill in the art at the time of this patent application, the fact that magnetic beads could be used for isolation of nucleic acids, or for the isolation of peptides and proteins is not new. Dynal has had products and methods for this since the eighties. However, technically and practically being able to combine the two methods in a single method, where neither protein nor nucleic acid components are damaged, is the novelty of the teachings of my patent application and the presently pending claims.

Lubenow specifically teaches the isolation of a fusion protein containing an engineered histidine tag, using IMAC. First, even if Lubenow were to be combined with Kleiber, as alleged by the Examiner and the ion exchange beads used in my claimed invention were to be substituted for the streptavidin beads for protein purification, an ion exchange resins in general, for protein isolation, would not work in a surrounding chemical solution containing high salt and

chaotropes, as described in the Kleiber method. Therefore, the combination of the Kleiber and Lubenow method would fail and would not be considered an obvious combination by a skilled person.

Furthermore, a person of skill in the art will recognize that the His-tagged protein of Lubenow is not a naturally-occurring protein in a sample, where combined isolation with a nucleic acid for the purposes I desired (obtaining a snapshot of a cell/sample in real-time) would be interesting or sufficient. In contrast to Lubenow, the currently claimed invention is not limited to specific affinity based protein purification but encompasses isolation of all nucleic acids and all proteins from cells from the same sample.

5c. I also cannot find that the buffers used by Kleiber and Lubenow are the same, as alleged by the Examiner. In particular, in my view, the detergents mentioned by Lubenow at column 4, lines 13-27, will not allow binding of nucleic acids to a glass surface as described by Kleiber. And as described earlier, the buffer of Kleiber, which has chaotropes, will denature proteins and not allow for isolation and analysis of protein.

5d. Neither Lubenow nor Kleiber mention sequential isolation of different components of a cell lysate, using different beads for one sample. They mention different matrices used separately, which was already well known in the art. If it was so obvious that a sequential isolation could be done, it is surprising that this is not mentioned in either Kleiber or Lubenow in clear terms showing how proteins and nucleic acids would be protected while the other was being isolated or that the Examiner has not found any other articles in their searches of literature that show the use of beads in sequential isolation of nucleic acids and proteins from a single sample.

6. Why “one of ordinary skill in the art”, at the time the invention was made, would not consider combining Kleiber and Lubenow and Schussler together to arrive at the claimed invention:

6a. Schussler describes isolation of nucleic acids and protein from small amount of tissue, by first isolating polyA<sup>+</sup>-RNA using Dynabeads Oligo(dT). DNA is then isolated from

supernatant by modified CTAB precipitation. Protein is isolated either from supernatant after DNA precipitation, or from supernatant/cell debris after polyA<sup>+</sup>-RNA removal (if DNA is not required).

Schussler however fails to use beads for the isolation of DNA and for the isolation of proteins. Schussler's proteins are described as "recovered" by precipitation and Schussler further describes that their proteins are dissolved from the pellet by "dilution" in a buffer. I do not see any description of proteins being "isolated using beads" as in the presently pending claims of my patent application. Thus, while Schussler may be describing isolating nucleic acids and proteins from a single sample, they do not use beads for each step. At best they use beads only for the mRNA isolation.

Furthermore, proteins can be concentrated by precipitation, but are not in a form where they can be handled in different ways, like easy buffer exchanges for downstream analyses as described in my present patent application.

If one of skill in the art were to combine the teachings of Schussler, as alleged by the Examiner, my ion exchanger bead surfaces would not bind proteins in the lysis buffer used in the Schussler publication for isolating mRNA using oligo(dT)-beads. So, the method described in Schussler would not easily be transferred to the magnetic particles used for separating proteins described in my patent application and currently pending claims.

The mRNA isolation by oligo(dT) beads in the Schussler method was followed by DNA and/or protein precipitation, which is very different from my claimed methods, which use beads for each purification.

7. Furthermore, my novel lysis buffers, described in my patent application are compatible with both stability of DNA, RNA and proteins, and the binding of DNA, RNA and protein to magnetic beads with different surface properties. Such buffers are not described by any of the cited art.

8. Thus, in summary my conclusions are as follows: One of ordinary skill in the art would not combine the teachings of the art cited by the Examiner since:

8a. Kleiber teaches away from the presently claimed invention as it teaches the use of chaotropic agents for isolating DNA, which would denature cellular proteins. In contrast to this, my claimed method teaches one of ordinary skill in the art to safely recover proteins for further analysis.

8b. Kleiber's description of streptavidin beads is only operational to isolate biotinylated proteins and not all proteins of a cell. In contrast, my invention is aimed at isolating as many (preferably all) protein and nucleic acid species from a cell sample to get a real time picture of a cell, accordingly, only isolating engineered biotinylated proteins is not the ultimate goal of the claimed invention.

8c. Furthermore, one of skill in the art will recognize that interchanging streptavidin beads with ion-exchange beads, as alleged by the Examiner, in Kleiber's buffer with chaotropic agents would not provide the results of the present claims.

8d. Lubenow teaches no more than isolating a fusion protein containing an engineered histidine tag, using immobilized metal ion affinity chromatography. Even if substituted with my ion exchange beads, ion exchange resins would not work in a surrounding chemical solution containing high salt and chaotropes, as described in the Kleiber method.

8e. Furthermore, if the detergents mentioned by Lubenow at column 4, lines 13-27, were to be used they would not allow binding of nucleic acids to a glass surface as described by Kleiber.

8f. Schussler describes no more than using oligo-dT beads to purify mRNA and fails to use beads for isolation of DNA and/or for further isolation of proteins.

8g. At least for these reasons, the combination of the teachings of Kleiber and Lubenow and Schussler would fail and would not be considered an obvious combination by a skilled person.

9. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made may jeopardize the validity of any patent issuing from the above captioned patent application.

Date: June 30, 2011

  
Marie Bosnes